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Effect of poly and mono-unsaturated fatty acids on stability and structure of recombinant S100A8/A9

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ABSTRACT

Recombinant pET 15b vectors containing the coding sequences S100A8 and S100A9 are expressed in *Escherichia coli* BL21 (DE3) and purified using Ni-NTA affinity chromatography. The structural changes of S100A8/A9 complex are analyzed upon interaction with poly/mono-unsaturated fatty acids (UFAs). The thermodynamic values, Gibbs free energy and the protein melting point, are obtained through thermal denaturation of protein both with and without UFAs by thermal scanning of protein emission using the fluorescence spectroscopy technique. The far-ultraviolet circular dichroism spectra show that all studied unsaturated fatty acids, including arachidonic, linoleic, alpha-linolenic and oleic acids, induce changes in the secondary structure of S100A8/A9 by reducing the α-helix and β-sheet structures. The tertiary structure of S100A8/A9 has fluctuations in the fluorescence emission spectra after the incubation of protein with UFAs. The blue-shift of emission maximum wavelength and the increase in fluorescence intensity of anilino naphthalene-8-sulfonic acid confirm that the partial unfolding is caused by the conformational changes in the tertiary structure in the presence of UFAs. The structural changes in S100A8/A9 and its lower stability in the presence of UFAs may be necessary for S100A8/A9 to play a biological role in the inflammatory milieu.

1. Introduction

S100A8 and S100A9 are mainly expressed in myeloid cells and perform a key role in the activities of these cells under inflammatory conditions. The S100A8/A9 complex, calprotectin, is a 36.5 kDa calcium- and zinc -binding protein in human neutrophil and granulocyte [1–3]. The serum level of S100A8/A9 heterodimer is increased in several human diseases, including acute inflammatory lesions and cystic fibrosis [4], rheumatoid arthritis [5] and formation and deposition of amyloids in the aging prostate [6]. The intervention of S100A8/A9 in mediating atherosclerosis [7] and cardiovascular inflammation has also been reported by several past studies [8–10]. Upon the elevation of intracellular calcium and activation of the protein kinase C, S100A8/A9 associates with non-covalent heterodimer [11], and is translocated from the cytosol to the cytoskeleton and the plasma membrane via a microtubule-dependent mechanism [12]. This heterodimer mediates calcium signals by binding to other intracellular proteins and regulating myeloid cell function [6], increasing the inflammation by influencing leukocyte trafficking [13]. In addition, the S100A8/A9 heterodimer is replaced in the detergent-resistant lipid structures of a membrane [14].

All eukaryotic organisms contain poly-unsaturated fatty acids (PUFAs) in their membrane lipids [15]. PUFAs are classified as *n*-3 and *n*-6 fatty acids based on the location of the last carbon–carbon double bond from the omega carbon at the end of the molecule [16]. Dietary PUFAs affect diverse physiological processes, impacting normal health and chronic diseases such as the regulation of plasma lipid level, cardiovascular diseases [17], inflammation and immune functions [18,19]. In fact, a balance between different poly-unsaturated fatty acids in the cell membranes has a desirable effect on the severity of the inflammation [20]. As the fatty acids have poor

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